EXPANDED MODEL FOR THE AEROBIC GROWTH OF *AEROMONAS HYDROPHILA*

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Received for Publication May 18, 1995
Accepted for Publication September 21, 1995

ABSTRACT

The previously published (Palumbo et al. *1991) response surface model for describing the influence of temperature, pH, NaCl, and sodium nitrite on the aerobic growth of* Aeromonas hydrophila *K144 in BHI broth has been expanded to incorporate additional data. The effects of the variables on* A. hydrophila *aerobic growth kinetics were modeled by response surface analysis using quadratic and cubic polynomial models of (1) natural logarithm transformation of both the Gompertz B and M parameters and the lag phase duration (LPD) and generation time (GT), and (2) the square root transformation of B and 1/M calculated from 268 cultures (212 of which supported growth) from 81 variable combinations. In addition, the six models generated also were subjected to backward elimination regression analysis to remove nonsignificant variables. Based on examination of the adjusted* R^2 *values of the resulting 12 models, three were selected for further evaluation by comparing their observed and predicted* T_{1000}*-values (time for a 1000-fold increase in number; this concept incorporates the influence of the variables on both lag and generation times), LPDs and GTs. Using this method of comparison and evaluation, models based on cubic polynomial, natural logarithm transformation of GT and LPD gave the best "first estimates" of the aerobic growth characteristics of* A. hydrophila.

INTRODUCTION

Today's consumer is demanding foods with fewer additives. This places increased reliance on refrigeration as the major factor limiting the growth of

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various foodborne pathogens and spoilage microorganisms in food products. However, refrigeration only slows but does not prevent the growth of psychrotrophic foodborne pathogens. The *Aeromonas hydrophila* group (motile aeromonads, mesophilic aeromonads) has received increased recognition as putative human foodborne pathogens. These psychrotrophic bacteria occur widely in the environment and on a wide variety of foods (Palumbo 1993). Refrigerated storage should be cold enough and of a duration short enough to ensure that this bacterium does not reach levels that represent a health risk. Alternatively, foods must rely on secondary barriers such as low pH, increased NaCl levels, and antimicrobials, or anaerobic (vacuum) packaging. This need to control pathogen growth while minimizing alterations in the food has stimulated the acquisition of data on the effects of multiple factors on microbial growth kinetics. Such data have been subsequently used to develop mathematical models to describe the effect of temperature and other variables on the growth of *Aeromonas hydrophila* (Palumbo *et al.* 1991, 1992; Hudson 1992).

In the predictive microbiology studies conducted in our laboratory, the bacterium has been cultured using standardized protocols. An advantage of that approach is that as additional experimentation is conducted, the results can be used to develop updated models that encompass wider variable ranges or provide more accurate estimates of variability. In this current study, we have appended additional data to earlier updated data sets to develop better models for the aerobic growth of *A. hydrophila*. We also used this as an opportunity to compare the effectiveness of models based on Gompertz parameters and derived values of generation (GT) and lag times (LT) and attempt to develop simplified models based on stepwise backward regression analysis. Models based on the derived values of GT and LPD will permit calculation of confidence intervals for the predicted values.

MATERIALS AND METHODS

Organism

Aeromonas hydrophila K144 was used in these studies. The bacterium was initially grown in 50 mL Brain Heart Infusion (BHI) broth (Difco; Detroit, MI) in a 250 mL flask. The flask was incubated for 18 h at 28C with agitation (150 rpm in a Psychrotherm Model G26; New Brunswick Scientific, Edison, NJ). Dilutions (made in 0.1% peptone water) were used to inoculate the experimental flasks (50 mL BHI broth in a 250 mL flask) to yield a zero time count of approximately 2×10^3 CFU/mL.

Experimental Procedure

Except where specifically indicated, the methods described in Palumbo *et al.* (1991, 1992) were followed. At intervals appropriate to the variable combination tested, samples were removed from the experimental flasks to determine viable count. This was done by surface plating onto tryptic soy agar (Difco) using a Spiral plater (Spiral Biotech, Bethesda, MD). Plates were incubated 24 to 36 h at 28C and then counted using a laser counting system (Spiral Biotech). Viable counts were converted to $\log_{10}$ values, and individual growth curves generated by the Gompertz equation (Gibson *et al.* 1987) in conjunction with Abacus (an iterative, nonlinear regression program; Damert 1994).

Protocol

The variables studied were: temperature (5 to 42C), pH (7.3 to 5.0 adjusted with HCl), NaCl (0.5 to 4.5%, w/v), and $NaNO_2$ (0 to 200 μg/mL). Sodium nitrite, a variable in the original data set (Palumbo *et al.* 1991), was added as a filter-sterilized solution after the medium was autoclaved. All cultures were incubated aerobically on a rotary shaker (150 rpm). Each variable combination was tested three times.

Analysis of Data

The expanded models were developed using the data set of Palumbo *et al.* (1991) which were obtained from 75 variable combinations and appending two additional data sets: a complete factorial design of two temperatures (5 and 19C), four pH levels (6.5, 6.0, 5.5 and 5.0), and two NaCl levels (0.5 and 2.0%) and 18 additional variable combinations (see Table 1 for the additional variable combinations included in this study). A total of 268 individual cultures were examined.

Quadratic and cubic response surface models for the Gompertz B and M values along with models for the derived kinetic parameters (LPD and GT) were generated for the 212 observations (268 total observations for the square root transformations) in the variables of temperature, pH, %NaCl, and $NaNO_2$ by the general linear models (SAS 1987, 1989). For the B and M values, the natural logarithm and square root transformations (B and 1/M) [use of the square root transformation permitted incorporation of variable combinations which did not support growth] were used; the natural logarithm transformation was also used for LPD and GT. Stepwise regression with a backwards elimination model selection method (Draper and Smith 1981) was used to simplify models by eliminating nonsignificant terms. The criterion for significance was set at $P>0.01$ level.

TABLE 1.

EFFECT OF CULTURE CONDITIONS ON THE OBSERVED GOMPERTZ PARAMETERS B AND M AND ON THE GENERATION TIME (GT) AND LAG PHASE DURATION (LPD) FOR THE AEROBIC GROWTH OF *A. HYDROPHILA* (CALCULATED FROM ACTUAL DATA BY THE GOMPERTZ EQUATION; AVERAGE OF THREE FLASKS).

Culture Variables			Calculated			
Temp,°C	pH	NaCl, %	B	M	GT	LPD
5	5.0	0.5		--NO GROWTH--		
5	5.0	2.0		--NO GROWTH--		
5	5.5	0.5	0.016	137.8	7.2	74.6
5	5.5	2.0		--NO GROWTH--		
5	5.5	2.5	0.013	563.0	8.8	486.0
5	6.0	0.5	0.06	81.1	2.1	64.6
5	6.0	2.0	0.046	105.5	2.5	83.8
5	6.5	0.5	0.087	79.1	1.4	67.5
5	6.5	2.0	0.064	73.3	2.0	55.8
12	5.5	2.0	0.108	77.9	1.2	68.7
12	5.5	3.0	0.054	177.2	2.5	156.0
12	6.0	2.0	0.132	53.6	0.8	32.7
12	6.0	3.0	0.049	101.1	2.7	80.5
19	5.0	0.5	0.024	134.8	5.2	92.3
19	5.0	2.0	0.081	49.7	1.5	37.4
19	5.5	0.5	0.168	27.7	0.7	21.8
19	5.5	2.0	0.099	18.8	1.1	8.4
19	5.5	2.5	0.08	31.6	1.5	19.1
19	6.0	0.5	0.283	22.2	0.4	18.6
19	6.0	2.0	0.13	14.7	0.8	7.0
19	6.0	2.5	0.234	27.4	0.5	22.5
19	6.5	0.5	0.375	19.3	0.3	16.6
19	6.5	2.0	0.184	13.8	0.6	8.3
28	5.0	2.5		--NO GROWTH--		
28	5.5	0.5	0.142	9.67	0.7	2.6
28	5.5	2.5	0.223	14.4	0.6	9.8
28	6.0	2.5	0.179	11.99	0.6	6.4
28	6.0	3.5	0.099	33.6	1.4	23.4
28	6.5	2.5	0.182	10.9	6.5	5.4
37	5.5	2.0	0.063	28.6	5.4	11.5
37	5.5	3.0		--NO GROWTH--		
37	6.0	2.0	0.178	11.1	0.6	5.4
37	6.0	3.0		--NO GROWTH--		
42	5.5	2.5		--NO GROWTH--		

Goodness of fit analyses of the models were performed on the SAS-generated ANOVA (SAS 1987, 1989) and other parameters. Maximum R^2 (R^2_{Max}) was calculated by the formula: (total sum of squares (TSS) - pure error sum of squares)/TSS. R^2_{Max} is constant for the data set and is thus independent of the model. The adjusted R^2 (R^2_{adj}) was then calculated using the formula: $R^2_{adj} = R^2/R^2_{Max}$.

An additional parameter, T_{1000} (time for a 1000-fold increase in number of viable cells), was also used to describe the influence of variables on the growth response of the bacterium and to compare the various models developed. This parameter combines the influence of the variables on both lag time and generation time [growth rate]. T_{1000} is calculated by the formula for our data set: $T_{1000} = (0.2169/B) + M$. B and M are calculated by the derived Gompertz formula: $Ln[-Ln(N-A/C)] = -B(t-M)$, where $N = A + 3$ and a C value of 6.71, the grand mean of C for all variables which supported growth, was assumed.

RESULTS AND DISCUSSION

The data used to generate the models obtained and evaluated represented a total of 268 individual growth curves (102 from this study and 166 from the study of Palumbo *et al.* 1991); 212 showed growth. The Gompertz parameters (B and M) and kinetic parameters (GT and LPD) for the additional culture variable combinations are shown in Table 1. Using response surface techniques, a total of 12 models were generated for the Gompertz B and M values and derived kinetic parameters. In addition, each of the twelve models was submitted to backward elimination to simplify through removal of nonsignificant terms. Based on the goodness of fit values, R^2_{adj}, only three models (VII, IX, and XI) were selected for further evaluation (Table 2). However, some general comments can be made: (1) as anticipated, cubic models for both the Gompertz parameters [B and M] and derived kinetic parameters [GT and LPD] provided a better fit of the data than quadratic models; and (2) the natural logarithm transformations of the Gompertz parameters gave better fits than the square root transformations. The models developed in this study had similar R^2 values to those developed from the smaller data base (Palumbo *et al.* 1991). The models for *A. hydrophila* developed by Hudson (1992), though based on optical density measurements, also had R^2 values similar to those calculated in this study. The F values obtained for models VII, IX, and XI are shown in Table 3. Many variables and the intercept, particularly for model XI, are highly significant ($P<0.001$).

The three models (models VII, IX, and XI; Table 2) were evaluated by another technique. Previous work from this laboratory (Buchanan and Bagi 1994; Zaika *et al.* 1994) has indicated that comparison of observed *versus*

TABLE 2.
GOODNESS OF FIT PARAMETERS FOR THE QUADRATIC AND CUBIC MODELS GENERATED FOR THE AEROBIC GROWTH OF *A. HYDROPHILA*.

Model Number	Model Type	Model Designation	Number of Observations	R^2_{adj}
VII	cubic	LnB	212	0.8548
	"	LnM	212	0.9513
IX	"	SqrtB	268	0.8274
	"	Sqrt(1/M)	268	0.8700
XI	"	LnGT	212	0.8455
	"	LnLPD	211	0.9466

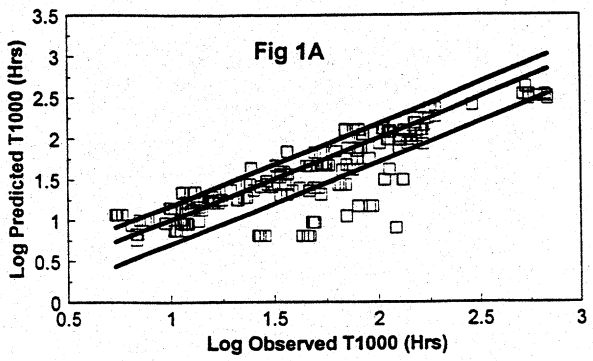
predicted T_{1000} values (time for a 1000-fold increase in population density) is a good means of evaluating the models since the term integrates the effects of the culture variables on LPD and GT. For the three (models VII, IX, and XI), predicted T_{1000} values were plotted against T_{1000} values calculated from the experimental data (Fig. 1a, 1b, and 1c, respectively). Based on the following considerations, model XI (cubic, natural logarithm transformation of GT and LPD) had the best agreement of predicted to observed (Fig. 1c): fewest values outside the $\pm$ 50% of observed, most values closest to line of identity, even distribution, and values outside the 50% confidence intervals were above the line of identity (thus model predictions would be conservative or fail-safe). Model XI was also compared to our previously published 'best choice' model (quadratic polynomial, natural logarithm transformation of Gompertz B and M values; Palumbo *et al.* 1991). A comparison of T_{1000} values showed that the new model (Model XI-this study) performed about the same as our previous model (plot not shown).

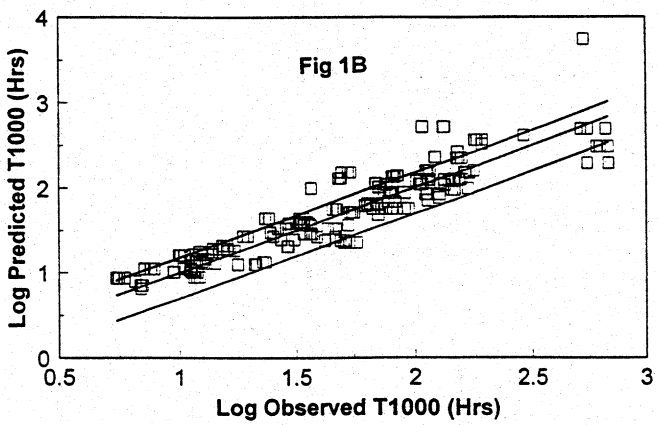
Final selection of a model to be utilized for a given bacterium is a function of various factors. The first is the bacterium for which the growth data are obtained. Hudson (1992) observed differences in the responses of two strains of *A. hydrophila* (the type strain ATCC 7966 and a food strain [isolated from cooked mussels]) to culture variables (temperature, pH, % NaCl). However, the type strain (ATCC 7966) was originally isolated from food (Popoff and Veron 1976). Thus, both models, though different, represent food isolates. The goodness of fit is also a means of choosing a model to describe the response of the bacterium of interest. In this study, model XI had the highest R^2_{adj} (Table 2) and comparison of observed to predicted T_{1000}

TABLE 3.
F VALUES FOR INDEPENDENT VARIABLES AND THEIR CROSS PRODUCTS FOR MODELS VII, IX, AND XI (SEE TABLE 2 FOR MODEL DESIGNATIONS)

	Model VII		Model IX		Model XI	
Variable	Ln B	Ln M	Sqrt B	Sqrt 1/M	Ln GT	Ln LPD
intercept	2.5	19.4*	3.0	6.6	1.8	20.0*
temp	4.0**	0.5	1.1	21.1*	0.6	1.7
pH	0.8	16.6*	1.7	6.8**	0.6	20.3*
NaCl	25.1*	2.7	6.9**	0.1	26.2*	0.2
NO_2	1.2	30.0*	4.2**	2.0	1.6	41.2*
temp*pH	3.2	0.1	1.4	20.4*	0.2	0.8
temp*NaCl	3.8	2.1	0.03	6.1**	2.8	4.0**
temp*NO_2	4.9**	17.3*	3.3	19.9*	1.0	4.7**
pH*NaCl	15.7*	0.04	3.4	0.5	16.9*	1.7
pH*NO_2	4.2**	16.2*	8.7**	3.5	4.3	31.8*
NaCl*NO_2	2.9	7.3**	10.0**	2.2	1.4	3.3
$temp^2$	0.1	33.5*	1.5	15.4*	0.4	38.4*
pH^2	0.1	15.9*	0.8	7.0**	0.1	21.6*
$NaCl^2$	16.3*	39.7*	11.4*	6.1**	16.7*	17.3*
NO_2^2	12.4*	7.4**	12.9*	8.0*	10.6**	3.0
temp*pH*NaCl	2.3	0.1	0.9	8.3*	1.3	0.3
temp*pH*NO_2	7.1**	22.1*	0.8	11.1*	1.5	6.4*
temp*NaCl*NO_2	2.8	0.01	14.8*	18.1*	2.9	0.2
pH*NaCl*NO_2	6.0**	17.7*	5.9*	1.1	3.1	9.0**
$temp^2$*pH	0.1	0.9	0.8	4.1**	2.2	1.0
$temp^2$*NaCl	0.1	23.1*	0.1	0.04	0.1	20.5*
$temp^2$*NO_2	1.1	0.5	4.1**	4.3**	0.1	0.6
pH^2*NaCl	8.9**	0.2	1.0	0.6	10.3*	2.3
pH^2*NO_2	7.9**	6.9**	11.5*	4.6**	7.1*	21.2*
temp*pH^2	3.3	0.1	1.2	20.9*	0.03	0.8
$NaCl^2$*NO_2	2.0	15.5	1.9	2.3	1.4	14.0*
pH*$NaCl^2$	8.3**	12.3*	11.9*	1.8	8.2**	2.9
$temp^2$ *NaCl	2.7	0.8	1.1	2.9	2.8	0.5
temp*NO_2^2	2.5	0.2	0.8	1.1	1.2	0.01
pH*NO_2^2	6.3**	6.7**	8.2**	7.7**	5.5*	4.9*
NaCL*NO_2^2	0.5	0.1	4.2	0.2	1.1	0.01
$temp^3$	1.6	80.0*	33.3*	146.5*	6.6**	77.6*
pH^3	0.1	15.1*	0.3	7.2*	0.02	22.8*
$NaCl^3$	3.5	15.4*	0.04	10.9*	3.8	11.1*
NO_2^3	0.5	0.1	0.02	0.1	0.1	0.8

F-values are based on type II sum of squares (SAS, 1987, 1989)
*$P < 0.001$
**$0.001 < P < 0.05$





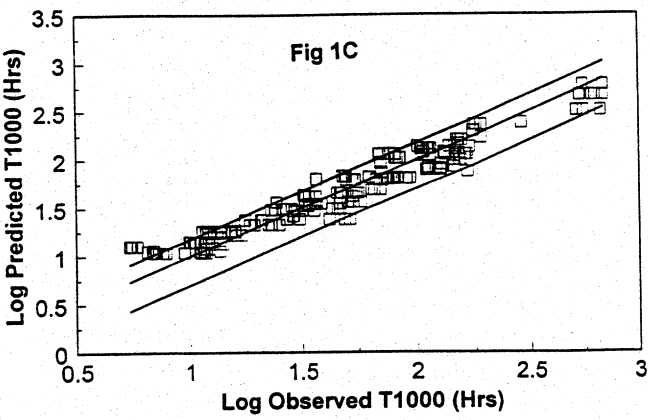


FIG. 1. COMPARISON OF OBSERVED AND PREDICTED T_{1000} VALUES FOR THREE MODELS (from Table 2)

Middle line is line of identity; top and bottom lines represent ± 50% of observed value. A. Model VII, B. Model IX, C. Model XI.

TABLE 4.
COMPARISON OF PREDICTED LPDs FOR *A. HYDROPHILA* USING VARIOUS MODELS

Culture Conditions			Predicted LPD			
Temp, °C	pH	NaCl, %	Previous Model*	Model XI, This Study	Type Strain Model	Hudson** Food Strain Model
5	5.0	0.5	493.3	303.0	42.2	3.5
5	5.0	2.0	930.0	477.8	80.4	11.8
5	5.5	0.5	208.6	105.6	75.2	7.4
5	5.5	2.0	378.3	128.8	141.5	17.8
5	5.5	2.5	491.5	274.3	202.8	31.3
5	6.0	0.5	110.8	84.5	94.1	10.2
5	6.0	2.0	198.0	89.7	182.0	19.7
5	6.5	0.5	75.4	89.2	95.4	10.6
5	6.5	2.0	136.8	92.7	197.4	18.1
12	5.5	2.0	72.8	50.2	14.6	3.9
12	5.5	3.0	138.7	200.3	32.4	8.9
12	6.0	2.0	42.0	35.0	16.6	4.2
12	6.0	3.0	83.0	108.2	34.8	7.8
19	5.0	0.5	21.3	53.2	1.5	0.6
19	5.0	2.0	45.2	48.0	3.3	1.3
19	5.5	0.5	9.7	16.9	2.0	0.9
19	5.5	2.0	21.6	12.4	3.3	1.3
19	5.5	2.5	31.1	23.1	4.8	1.8
19	6.0	0.5	5.8	13.3	2.0	0.9
19	6.0	2.0	14.2	8.9	3.5	1.3
19	6.0	2.5	20.9	15.0	4.9	1.7
19	6.5	0.5	4.8	14.9	1.7	0.9
19	6.5	2.0	12.9	10.2	3.3	1.2
28	5.0	2.5	25.3	31.7	1.4	1.0
28	5.5	0.5	2.9	3.4	0.7	0.3
28	5.5	2.5	14.8	7.4	1.7	0.9
28	6.0	2.5	12.0	5.2	1.6	0.7
28	6.0	3.5	31.8	22.5	4.4	1.2
28	6.5	2.5	12.8	6.5	1.5	0.6
37	5.5	2.0	9.8	7.7	0.7	0.5
37	5.5	3.0	27.9	73.3	1.7	1.5
37	6.0	2.0	9.3	6.8	0.6	0.4
37	6.0	3.0	25.3	53.0	1.6	1.0
42	5.5	2.5	24.6	192.1	---***	---***

*Quadratic model, natural logarithm of Gompertz B and M values, Palumbo et al (1992).
**Cubic polynomial model of Hudson (1992).
***Can not be calculated because temperature is beyond range of model.

values indicated that model XI had the best fit of the models evaluated (Fig. 1a, 1b, and 1c). Ability to predict responses of the bacterium to changes in culture conditions is another basis on which to select a model. We compared our previous model (Palumbo *et al.* 1991), model XI (this study), and the two models of Hudson (1992) to predict LPDs for the culture variables in Table 1. These comparisons are presented in Table 4. Comparison of the calculated LPDs (last column, Table 1) with those predicted by the different models indicated that model XI (this study) yielded improved predictions in more than half of the variable combinations compared to our previous model and in only a limited number of variables was the previous model better. Further, the models developed by Hudson (1992) considerably underestimated LPDs for essentially all variable combinations. This last point suggest that our models best fit *A. hydrophila* K144 while Hudson's models best fit his strains. Perhaps the *A. hydrophila* group is too large and varied genotypically to have a single growth model applicable for all strains. Another approach might be to utilize a cocktail of several strains and develop the growth kinetics from this mixture of strains. This approach has been utilized in our laboratory for various bacteria, including *Escherichia coli* (Buchanan and Klawitter 1992).

As indicated, development of models based on the kinetic parameters (GT and LPD) allowed calculation of the $\pm 95\%$ confidence intervals for predicted values for these two parameters. Using SAS, confidence intervals for GT and LPD from model XI were generated and used in a spreadsheet format. This then permitted us to determine the responses of the bacterium to changes in one culture condition (storage temperature) for selected foods: (1) fresh beef or fish (pH 5.8, 0.5% NaCl, and no nitrite) and (2) a lightly salted, fermented cured product (pH 5.1, 2% NaCl, and 10 ppm nitrite). These responses are shown in Fig. 2. In general, the predicted responses and $\pm 95\%$ confidence intervals are tighter at the higher storage temperatures. Since *A. hydrophila* K144 grows faster as the temperature increases up to 37C, the closer fit may reflect this. It could also result from more of the data points used to generate the models being gathered at higher temperatures.

From Table 4, it can also be seen that model XI gave somewhat improved performance over our previous model. In summary, based on multiple factors, model XI (full cubic model, natural logarithm transformation of LPD and GT; Table 5) represents the current best "first estimates" of the aerobic growth characteristics of *A. hydrophila* K144.

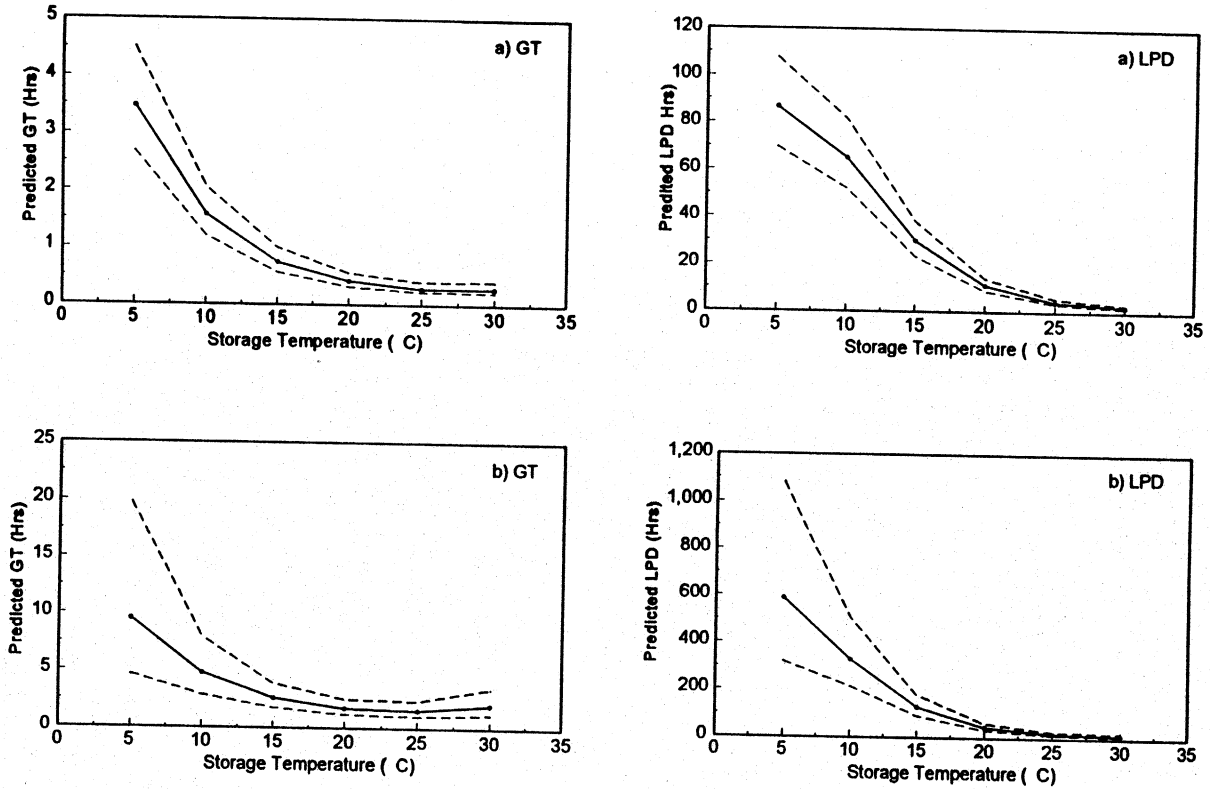


FIG. 2. PREDICTED RESPONSES (GT and LPD) OF *A. hydrophila* TO CHANGES IN STORAGE TEMPERATURE USING MODEL XI
Solid line is predicted parameter, top dashed line +95% confidence interval of the predicted, and bottom dashed line is -95% confidence interval of predicted. a. Fresh beef or fish (pH 5.8, 0.5% Nacl, no nitrite). b. A lightly salted, fermented cured product (pH 5.1, 2% NaCl, 10 ppm).

TABLE 5.
EXPANDED RESPONSE SURFACE MODEL FOR THE AEROBIC GROWTH OF *A. HYDROPHILA* K144 IN BHI (MODEL XI-NATURAL LOGARITHM TRANSFORMATION OF GT AND LPD, CUBIC): [T = TEMPERATURE IN °C, P = PH, S = % NACL, AND N = SODIUM NITRITE IN MG/L]

$$\text{Ln GT} = 64.381367 - 0.497299*T - 17.467024*P - 25.187096*S - 0.173627*N + 0.074474*T*P + 0.104694*T*S - 0.001127*T*N + 6.232554*P*S + 0.088402*P*N - 0.014711*S*N + 0.00313*T^2 + 1.019777*P^2 + 2.894051*S^2 - 0.000599*N^2 - 0.011477*T*P*S + 0.000216*T*P*N - 0.000195*T*S*N + 0.003267*P*S*N - 0.001025*T^2*P + 0.000182*T^2*S - 0.0000031*T^2*N - 0.401467*P^2*S - 0.009042*P^2*N - 0.002714*T*P^2 - 0.001235*S^2*N - 0.285522*P*S^2 - 0.00876*T*S^2 + 0.0000022*T*N^2 + 0.000064*P*N^2 + 0.0000208*S*N^2 + 0.000126*T^3 + 0.028696*P^3 - 0.140881*S^3 + 0.00000009*N^3.$$

$$\text{Ln LPD} = 179.638116 + 0.700233*T - 84.797069*P + 1.671752*S + 0.751659*N - 0.144674*T*P - 0.105946*T*S - 0.002090*T*N - 1.656374*P*S - 0.201880*P*N - 0.018798*S*N - 0.025179*T^2 + 13.707846*P^2 + 2.461368*S^2 - 0.000268*N^2 + 0.004437*T*P*S + 0.000368*T*P*N + 0.000044*T*S*N + 0.004662*P*S*N + 0.000586*T^2*P + 0.002077*T^2*S - 0.0000087*T^2*N + 0.156729*P^2*S + 0.013051*P^2*N + 0.010958*T*P^2 - 0.003273*S^2*N - 0.141542*P*S^2 + 0.002939*T*S^2 + 0.0000002*T*N^2 + 0.0000459*P*N^2 - 0.0000013*S*N^2 + 0.000364*T^3 - 0.738128*P^3 - 0.201627*S^3 - 0.0000002*N^3.$$

REFERENCES

BUCHANAN, R.L. and BAGI, L.K. 1994. Expansion of response surface models for the growth of *Escherichia coli* O157:H7 to include sodium nitrite as a variable. Intl. J. Food Microbiol. *23*, 317–332.

BUCHANAN, R.L. and KLAWITTER, L.A. 1992. The effect of incubation temperature, initial pH, and sodium chloride on the growth kinetics of *Escherichia coli* O157:H7. Food Microbiol. *9*, 185–196.

DAMERT, W. 1994. ABACUS: Interactive program for nonlinear regression analysis. QCPE Bulletin *14*, 61.

DRAPER, N.R. and SMITH, H. 1981. Applied regression analysis, 2nd ed., pp. 305–307, John Wiley & Sons, New York.

GIBSON, A.M., BRATCHELL, N. and ROBERTS, T.A. 1987. The effect of sodium chloride and temperature on the rate and extent of growth of *Clostridium botulinum* type A in pasteurized pork slurry. J. Appl. Bacteriol. *62*, 479–490.

HUDSON, J.A. 1992. Construction of and comparisons between response surface models for *Aeromonas hydrophila* ATCC 7966 and a food isolate under aerobic conditions. J. Food Prot. *55*, 968–972.

PALUMBO, S.A. 1993. The occurrence and significance of organisms of the *Aeromonas hydrophila* group in food and water. Med Microbiol Lett *2*, 339-346.

PALUMBO, S.A., WILLIAMS, A.C., BUCHANAN, R.L. and PHILLIPS, J.G. 1991. Model for the aerobic growth of *Aeromonas hydrophila* K144. J. Food Prot. *54*, 429-435.

PALUMBO, S.A., WILLIAMS, A.C., BUCHANAN, R.L. and PHILLIPS, J.G. 1992. Model for the anaerobic growth of *Aeromonas hydrophila* K144. J. Food Prot. *55*, 260-265.

POPOFF, M. and VERON, M. 1976. A taxonomic study of the *Aeromonas hydrophila-Aeromonas punctata* group. J. Gen. Microbiol. *94*, 11-22.

SAS INSTITUTE, INC. 1987. SAS/STAT Guide for Personal Computers, Version 6 Ed. SAS Institute Inc., Cary, NC.

SAS INSTITUTE, INC. 1989. SAS/STAT Users' Guide Version 6, Fourth ed., Vol. 1. SAS Institute, Inc., Cary, NC.

ZAIKA, L.L., MOULDEN, E., WEIMER, L., PHILLIPS, J.G. and BUCHANAN, R.L. 1994. Model for the combined effect of temperature, initial pH, sodium chloride and sodium nitrite concentration on anaerobic growth of *Shigella flexneri*. Intl. J. Food Microbiol. *23*, 345-358.